MICROPARTICLES FOR DELIVERY OF NUCLEIC ACID

BACKGROUND OF THE INVENTION

This invention relates to methods of delivering nucleic acids into cells.

Gene therapy is a highly promising technique for treatment of hereditary diseases, e.g., cystic fibrosis. Gene therapy can also be used when expression of gene products from genes which are not naturally found in the host cells is desired, for example, from genes encoding cytotoxic proteins targeted for expression in cancer cells.

Gene therapy can fall into several categories. It is sometimes desirable to replace a defective gene for the entire lifespan of a mammal, as in the case of an inherited disease such as cystic fibrosis, phenylketonuria, or severe combined immunodeficiency disease (SCID). In other cases, one may wish to treat a mammal with a gene that will express a therapeutic polypeptide for a limited amount of time, e.g., during an infection. Nucleic acids in the form of antisense oligonucleotides or ribozymes are also used therapeutically. Moreover, polypeptides encoded by nucleic acids can be effective stimulators of the immune response in mammals.

Various techniques have been used for introducing genes into cells, including infection with viral vectors, biolistic transfer, injection of "naked" DNA (U.S. Pat. No. 5,580,859), and delivery via liposomes or polymeric particles.

SUMMARY OF THE INVENTION

The invention is based on the discovery that microparticles containing nucleic acids having an appropriate size for phagocytosis can be made without adversely affecting nucleic acid integrity. These microparticles are highly effective vehicles for the delivery of polynucleotides into phagocytic cells.

In general, the invention features a preparation of microparticles, each of which includes a polymeric matrix and a nucleic acid expression vector. The polymeric matrix includes one or more synthetic polymers having a solubility in water of less than about 1 mg/l; in the present context, synthetic is defined as non-naturally occurring. At least 90% of the microparticles have a diameter less than about 100 microns. The nucleic acid is either RNA, at least 50% (and preferably at least 70% or even 80%) of which is in the form of closed circles, or circular DNA plasmid molecules, at least 50% (and preferably at least 70% or even 80%) of which are supercoiled. In some cases, it is desirable for at least 90% of the microparticles to have a diameter less than about 20 microns, and preferably less than about 11 microns.

Another embodiment of the invention features a microparticle less than about 20 microns in diameter, including a polymeric matrix and nucleic acid. The polymeric matrix is made from one or more synthetic polymers having a solubility in water of less than about 1 mg/l. At least 50% (and preferably at least 70% or even 80%) of the nucleic acid molecules are in the form of supercoiled DNA.

The polymeric matrix can be biodegradable. Biodegradable is used here to mean that the polymers degrade over time into compounds which are known to be cleared from the host cells by normal metabolic pathways. Generally, a biodegradable polymer will be substantially metabolized within about 1 month after injection into a patient, and certainly within about 2 years. In certain cases, the polymeric matrix can be made of a single synthetic, biodegradable copolymer, e.g., poly-lactic-co-glycolic acid (PLGA).

The ratio of lactic acid to glycolic acid in the copolymer can be within the range of about 1:2 to about 4:1 by weight, preferably within the range of about 1:1 to about 2:1 by weight, and most preferably about 65:35 by weight. In some cases, the polymeric matrix also includes a targeting molecule such as a ligand, receptor, or antibody, to increase the specificity of the microparticle for a given cell type or tissue type.

For certain applications, the microparticle has a diameter of less than about 11 microns. The microparticle can be suspended in an aqueous solution (e.g., for delivery by injection) or can be in the form of a dry solid (e.g., for storage or for delivery via inhalation or implantation). The nucleic acid can be an expression control sequence operatively linked to a coding sequence. Expression control sequences include, for example, any nucleic acid sequences known to regulate transcription or translation, such as promoters, enhancers, or silencers. In preferred examples, at least 60% or 70% of the DNA is supercoiled. More preferably, at least 80% is supercoiled.

In another embodiment, the invention features a microparticle less than about 20 microns in diameter, including a polymeric matrix and a nucleic acid molecule (preferably in closed, circular form), wherein the nucleic acid molecule includes an expression control sequence operatively linked to a coding sequence. The expression product encoded by the coding sequence can be a polypeptide at least 7 amino acids in length, having a sequence essentially identical to the sequence of either a fragment of a naturally-occurring mammalian protein or a fragment of a naturally-occurring protein from an agent which infects or otherwise harms a mammal; or a peptide having a length and sequence which permit it to bind to an MHC class I or II molecule. Examples are set forth in WO 94/04171, herein incorporated by reference.

Essentially identical in the context of a DNA or polypeptide sequence is defined here to mean differing no more than 25% from the naturally occurring sequence, when the closest possible alignment is made with the reference sequence and where the differences do not adversely affect the desired function of the DNA or polypeptide in the methods of the invention. The phrase fragment of a protein is used to denote anything less than the whole protein.

The polypeptide and the peptide can each be linked to a trafficking sequence. The term "trafficking sequence" refers to an amino acid sequence which causes a polypeptide to which it is fused to be transported to a specific compartment of the cell, e.g., the nucleus, endoplasmic reticulum, a lysosome, or an endosome.

In the embodiment where the expression product includes a peptide having a length and sequence which permit it to bind an MHC class I or II molecule, the expression product is typically immunogenic. The expression product can have an amino acid sequence that differs from the sequence of a naturally occurring protein recognized by a T cell in the identity of not more than 25% of its amino acid residues, provided that it can still be recognized by the same T cell and can alter the cytokine secretion profile of the T cell (i.e., an "altered peptide ligand").

Examples of expression products include amino acid sequences at least 50% identical to the sequence of a fragment of myelin basic protein (MBP), proteolipid protein (PLP), invariant chain, GAD65, islet cell antigen, desmoglein, α-crystallin, or β-crystallin, where the fragment can bind the MHC class II molecule. Table 1 lists many of such expression products that are thought to be involved in